The relationship between powdery mildew (Sphaerotheca fuliginea) resistance and leaf chlorosis sensitivity in cucumber (Cucumis sativus) studied in single seed descent lines

Zijlstra, S.; Jansen, R.C.; Groot, S.P.C.

Published in:
Euphytica

DOI:
10.1007/BF00025433

Publication date:
1995

Euphytica **81:** 193–198, 1995.

The relationship between powdery mildew (*Sphaerotheca fuliginea*) resistance and leaf chlorosis sensitivity in cucumber (*Cucumis sativus*) studied in single seed descent lines

S. Zijlstra, R.C. Jansen & S.P.C. Groot
Center for Plant Breeding and Reproduction Research (CPRO-DLO), Department of Vegetable and Fruit Crops, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Received 30 May 1994; accepted 24 November 1994

Key words: cucumber, *Cucumis sativus*, leaf chlorosis, powdery mildew, single seed descent, *Sphaerotheca fuliginea*

Summary

The genetic relation between powdery mildew resistance and sensitivity for leaf chlorosis of glasshouse cucumber was investigated. The powdery mildew resistant, leaf chlorosis sensitive hybrid variety 'Profito' was crossed with the powdery mildew susceptible, non chlorosis sensitive hybrid variety 'Corona'. Forty four F_6 inbred lines of this cross, produced by single seed descent, were tested for powdery mildew resistance (PMR) and leaf chlorosis sensitivity (LCS). PMR and LCS were positively correlated (r = 0.59). One or more of the PMR genes probably causes LCS as a pleiotropic effect or is closely linked to LCS, but also other factors, not genetically linked to PMR can cause LCS. Five F_6 lines combined a significantly higher level of PMR than 'Corona' with a significantly lower LCS than 'Profito'. Three of these lines could hardly be distinguished from 'Corona' for the level of LCS.

Abbreviations: LCS – leaf chlorosis sensitivity, PMR – powdery mildew resistance, SSD – Single Seed Descent

Introduction

In cucumber (*Cucumis sativus* L.) a high level of partial resistance to the powdery mildews *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. (Kooistra, 1968) and *Erysiphe cichoracearum* DC. ex Mérat emend. Salm (Leppik et al., 1964) has been known for decades. In The Netherlands varieties with powdery mildew resistance (PMR) have not been very successful, because they suffer from leaf chlorosis during periods of low light intensity and short day length (Groot et al., 1992). Such periods occur in autumn, winter and early spring in the Netherlands. Previous research indicated that partial resistance to *Sphaerotheca fuliginea*, the common powdery mildew species in glasshouse cultivation (Zijlstra & Groot, 1992), is based on unlinked segregating recessive genes (Kooistra, 1968). Genes conferring leaf chlorosis sensitivity (LCS) could be linked to one or more of these PMR genes. We investigated the involvement of the PMR genes in 'Profito' in LCS.

In this paper we report the genetic variation for LCS and PMR and about the correlation of LCS with PMR in an F_2 population, from a cross between 'Corona' (PM susceptible, no LCS) and 'Profito' (high level of PMR, high LCS). To study the relationship between LCS and PMR a set of homozygous cucumber lines was produced by single seed descent. In these lines both the level of PMR and of LCS was determined.

Material and methods

Plant material

Two Dutch cucumber hybrid varieties were chosen for this study. 'Corona' is a powdery mildew susceptible and 'Profito' a highly partial resistant variety. 'Profito' suffers from leaf chlorosis when cultivated in autumn, winter and early spring (Groot et al., 1992), resulting in reduced fruit yield. 'Corona' is not sensitive to leaf chlorosis.

Both 'Corona' and 'Profito' exclusively produce female flowers. To produce male flowers for making the crosses and the self pollinations, three week old seedlings were sprayed with a suspension of 5.83 g/l argylene, containing 8% Sodium Silver Thiosulphate (Den Nijs & Visser 1980).

'Corona' was crossed with 'Profito' and two F_1 plants, from which one plant was sprayed with argylene to produce male flowers, were crossed to obtain F_2 seeds. Using this method for F_2 production we assumed the two parents Corona and Profito to be homozygous for PMR and LCS. The single seed descent (SSD) method was used to produce homozygous lines (Brim, 1966). 44 F_2 plants were raised and self pollinated to obtain F_3 seeds. One F_3 seed from each F_2 plant was raised to produce, after self pollination, the next generation. This process was repeated until the F_6 generation. Assuming that the PMR of 'Profito' is based on maximally three unlinked segregating recessive genes (Kooistra, 1968), it can be expected that all eight possible homozygous combinations of the PMR genes are present in the set of 44 F_6 lines ($P > 0.95$) (Jansen & Jansen, 1990).

PMR and LCS were tested in 2 glasshouse experiments, the first experiment contained all 44 F_6 lines and the crossing parents. The second experiment was carried out with 15 selected F_6 lines, covering the whole range observed for both PMR and LCS from the first set.

In the mildew tests the crossing parents 'Corona' and 'Profito' and a control set of the genotypes PI 200815 (low resistance level), 'Natsufushinari' (moderate resistance level) and 'NPI' (a crossing of Natsufushinari with PI 200815, showing a high resistance level, based on three recessive genes according to Kooistra (1968)) were included. In the chlorosis tests the crossing parents 'Corona' and 'Profito' were included as controls.

Table 1. Powdery mildew and leaf chlorosis scores

Classification of powdery mildew severity	
Leaf	0 = no or very few sporulating colonies
	1 = more and clearly sporulating colonies
	2 = many sporulating colonies
Stems and petioles	0 = no or very few sporulating colonies
	1 = more sporulating colonies
Hypocotyl	0 = no or very few sporulating colonies
	1 = more sporulating colonies

Classification of leaf chlorosis severity
0 = no symptoms
1 = leaf with one or a few chlorotic spots
2 = leaf chlorotic between the veins
3 = major part of leaf chlorotic, sometimes with pale spots
4 = leaf entirely chlorotic with necrotic spots

Mildew tests

The first mildew test took place in the autumn of 1990. The second test was performed in the spring of 1992. The minimum air temperature in the glasshouse was set at 23° C at day and 19° C at night, with a relative air humidity maintained at least 70%. The plants were arranged on a growing table with ± 14 plants per m^2 according to a randomized block design with three plants per plot and eight replicates. The inoculum of *Sphaeroteca fuliginea* originated from a monospore culture, maintained on the susceptible cultivar Vetobit on a culture medium (Murashige & Skoog, 1962) in isolated glass pots, and was multiplied once on the susceptible cultivar in the glasshouse before use as inoculum. In the first test a spore suspension of ± 10^4 spores ml^{-1} was used for inoculation. Cotyledons of six days old seedlings were inoculated with a plant sprayer and inoculation was repeated one and two weeks later. In the second test the cotyledons and the first leaf of 14 days old seedlings were inoculated with a suspension of 10^3 spores ml^{-1}. The inoculation was repeated ten days later with a suspension of 3×10^3 spores ml^{-1}. For each inoculation, a suspension density of ± 35 ml/m^2 was supplied.

Plants were scored for PMR 33 days after first inoculation. Each plant was judged for the level of disease incidence on leaves, stem plus petioles and hypocotyl separately. The classification method for PMR is shown in Table 1.

Chlorosis tests

The first chlorosis test was performed during the winter of 1990–1991, the second test in the winter of 1991–1992. Plants were sown on 16 and 15 November, respectively for the first and second test and raised in rockwool cubes. During the raising period the plants received a standard nutrition solution, recommended by the Glasshouse Crops Research and Experimental Station (Naaldwijk, The Netherlands), extra CO_2 to 500 ppm and SON-T lamps (Philips, 25 Watts m^2 visi-

ble radiation) during 10 hours per day. The plants were transplanted on rockwool bags at day 32 and day 26 after sowing for test one and test two, respectively. Plants were arranged according a randomized block design with 1 plant per plot and 11 respectively 13 replicates for the first and second test. The minimum air temperature for both tests was set at 23° C at day and 19° C at night. The standard nutrition solution was modified for P and N to increase the LCS (Groot et al., 1992). Extra P nutrition, 3.0 mM instead of 1.25 mM, was given by replacing 10% of the KNO_3 with KH_2PO_4. The nutrient solution was applied by drip irrigation.

The top of the main shoot of the plants was cut at ± 2 m height and two side shoots were allowed to grow. Chlorosis severity was scored at day 108 for test one and at day 116 for test two, when differences between the parents 'Corona' and 'Profito' were very pronounced. From each plant the leaves 11 to 16 on the main stem, counted from the first true leaf upwards, and the first 6 leaves from one, randomly chosen, side shoot were scored. The classification method of chlorosis severity is shown in Table 1.

Analysis of mildew and chlorosis scores

In the mildew test for each plant the scores of the different plant parts are summed, so that a score between 0 and 4 is obtained (Table 1).

Standard analysis of variance (ANOVA) cannot be used to analyze the scores from our mildew and chlorosis tests, because the statistical assumptions for ANOVA may be seriously violated. A threshold model for ordered categorical data may provide a suitable method for analyzing PMR and LCS scores in cucumber. The threshold model assumes the presence of an underlying, continuous variable γ that is related to the observed variate. Categorization is thought to arise from partitioning this underlying scale by four thresholds (θ_1, θ_2, θ_3 and θ_4, Fig. 1C) which are assumed to be the same for all genotypes. A plant is assigned to category 0 of the ordinal scale if the value of γ is less than or equal to the threshold θ_1. A plant is assigned to category 1 if its value of γ lies between θ_1 and θ_2, and so on. Finally, the plant is assigned to category 4 if its value of γ is larger than θ_4.

In the chlorosis test for each leaf of a plant a score between 0 and 4 is scored (Table 1). Next for each plant the number of leaves in each scoring class was counted; these values were analyzed. A Genstat procedure was used to fit the models to the data. See Straathof et al. (1993) for a full description of threshold models for disease ratings.

Results and discussion

Mildew tests

The PMR levels of the SSD lines in the first test are summarized in Fig. 1A. Twenty two of the lines were significantly more resistant than 'Corona', which had a score of 9. Seventeen lines had the same susceptibility as 'Corona'. Five lines proved to be significantly more susceptible than 'Corona', which is exceptional, since 'Corona' is known as a highly susceptible variety. Thirty two lines were significantly more susceptible than 'Profito' (score of 0). Twelve lines had the same level of resistance as 'Profito'. One line (score of - 2) exhibit a higher resistance, but did not significantly differ from 'Profito'. Ten lines were significantly more resistant than 'Corona' and significantly more susceptible than 'Profito'. The mildew sporulation on these lines was, compared to 'Corona', depressed on the leaves with sometimes a single sporulating colony on the petioles or the stem and no symptoms on the hypocotyl.

When the frequency distribution of Fig. 1A is considered as two-peaked, with a threshold value of 5 dividing highly resistant versus weakly resistant and susceptible lines, 15 lines are estimated to be highly resistant (scores below 5) and 29 lines as having a low level or no PMR (scores above 5). It can not be excluded that some lines are still heterozygous for PMR. Because PMR is recessive, these lines will be classified as susceptible, thus causing the surplus of susceptible lines in our results. When two unlinked loci are involved a maximum of 6 lines (P = 0.95) is expected to be still heterozygous for one or two genes (Jansen & Jansen, 1990). However, even if all lines were homozygous for the PMR genes, the proportion resistant : susceptible is close to the ratio 1 : 1 (χ^2 = 4.4). This suggests one major gene for PMR of 'Profito'. The PMR of 'Profito' is similar to the PMR of 'Natsufushinari' in both tests (Fig. 2A). According to Kooistra (1968) the PMR of 'Natsufushinari' is based on two recessive genes, whereas Shanmugasundaram et al. (1971) suggested one major recessive gene and two minor genes (one dominant and one recessive). From the present study it seems most likely that one major gene plus one or some modifyer genes determine the PMR of 'Profito'.

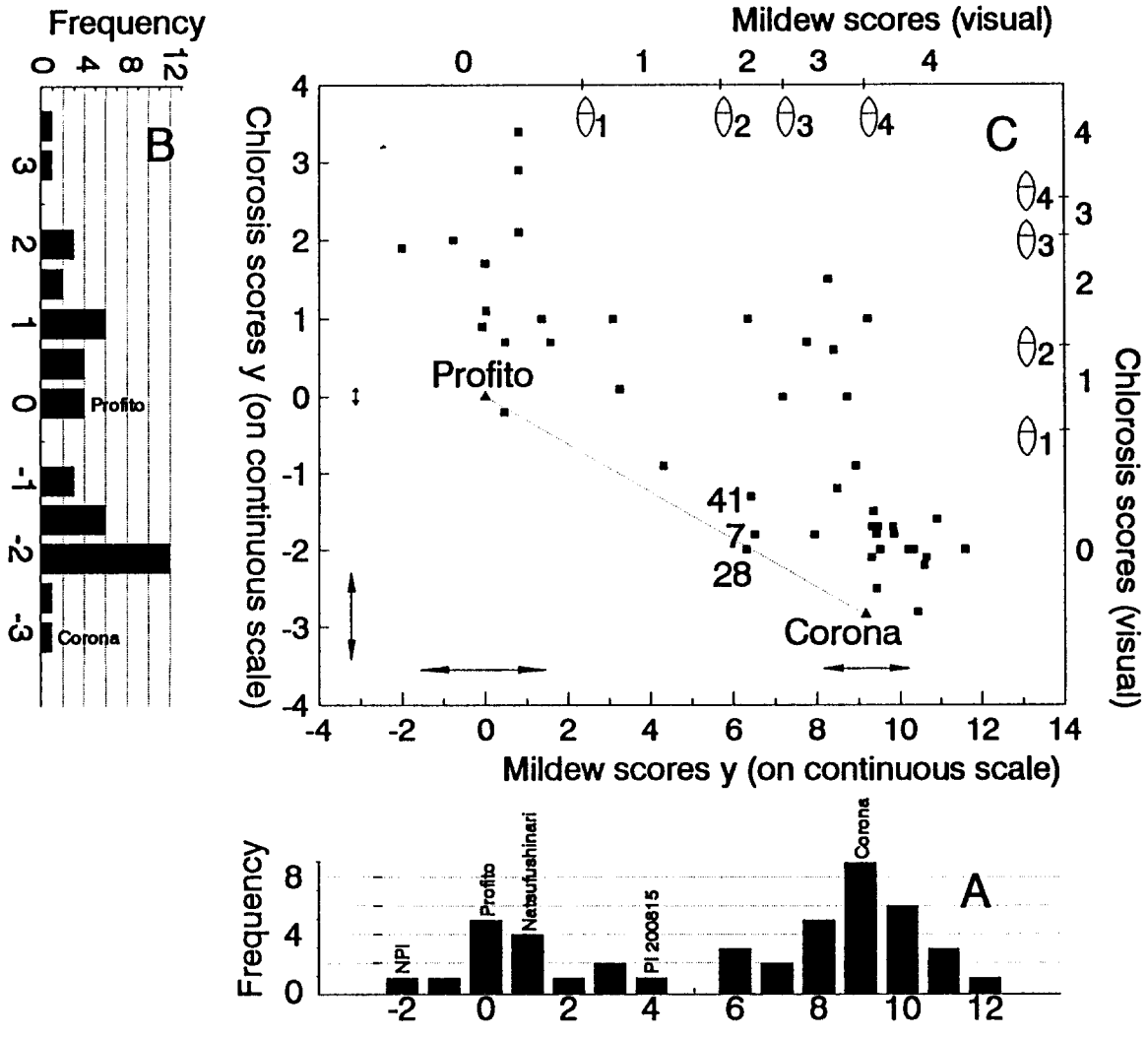

Fig. 1. Average values (scores) per line and crossing parent of powdery mildew and leaf chlorosis tests (Experiment 1).
A: frequency of the SSD lines per calculated powdery mildew scores (on continuous scale) relative to 'Profito' (score of 0). Names of the parents and control genotypes are located above their mildew and chlorosis score, respectively.
B: identical for leaf chlorosis.
C: relation between the powdery mildew and leaf chlorosis scores of the SSD lines including 'Corona' and 'Profito'. Scales for visual scores are included on the top and right of the figure; double headed arrows indicate which SSD lines do not significantly differ from 'Profito' and 'Corona', respectively.

The results of the second test were very much in accordance with those of the first test (r = 0.97, Fig. 2A). The average level of disease was higher in spite of the fact that the inoculation was done with a lower concentration of spores. This may be explained by the effect of the season.

Chlorosis tests

The first symptoms of chlorosis were visible 52 days after sowing and the amount of chlorosis increased with time. Differences between the parents 'Corona' and 'Profito' as well as between SSD lines were very pronounced in March, 108 days after sowing. These results are summarized in Fig. 1B.

Twenty four SSD lines were, together with 'Corona' (score of - 3), significantly less chlorotic than

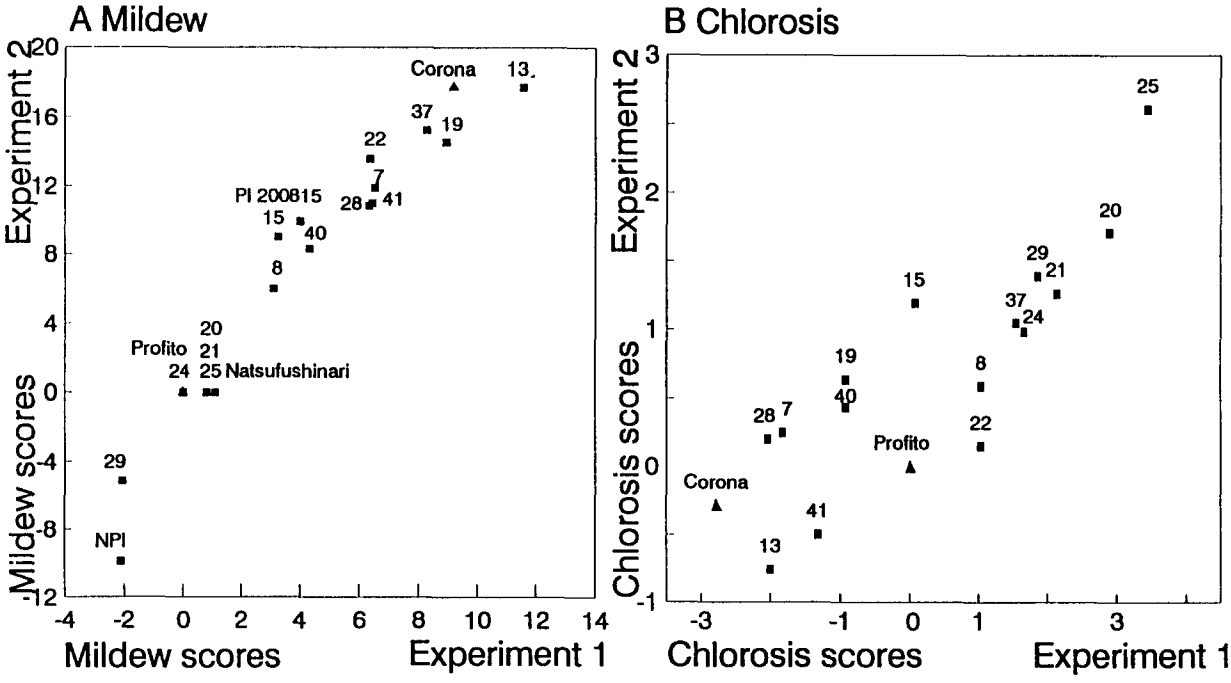

Fig. 2. Relation between powdery mildew (A) and leaf chlorosis (B) scores in the two experiments (calculated values per line or control genotype relative to 'Profito' (score of 0, 0)).

'Profito' (score of 0). Twenty of these were significantly more chlorotic than 'Corona' and four similar to 'Corona'. Sixteen lines were significantly more chlorotic than 'Profito' although 'Profito' was the sensitive parent in the cross.

The results of the second chlorosis test highly correlate with the results of the first test (r = 0.86, Fig. 2B). The average level of chlorosis was lower than in the first test. Leaves of the side shoot were somewhat more chlorotic compared to those of the main stem. Although the leaves on the side shoot of 'Profito' proved to be significantly more LCS than those of 'Corona', the leaves on the main stem of 'Profito' did not differ from those of 'Corona', resulting in an overall LCS score of 'Profito' which did not differ significantly from 'Corona' in the second test. These variable results between two tests and between leaves of the main stem and a side shoot within one test, reflect the variable expression of LCS and indicates that selection for LCS will be difficult and time consuming. Observed differences between tests may be the result of small differences in light intensity or duration of the tests (Groot et al., 1992).

In spite of these difficulties the correlation between the scores for LCS of leaves from the main stem and those from the side shoot was significant for both the first (r = 0.94) and the second (r = 0.77) test.

Relation between PMR and LCS

Most interesting of the experimental results is the putative genetic linkage between the levels of PMR and of LCS from the SSD lines. Figure 1C presents the relationship between both characters in the first experiment. A significant positive correlation between the level of PMR and LCS was found (r = 0.59). The average level of LCS of all SSD lines lies far above the line 'Corona' – 'Profito'. From the twenty powdery mildew susceptible lines, thirteen lines proved to be significantly more LCS than 'Corona' and two lines were even significantly more LCS than 'Profito'. This indicates that LCS can have different genetic causes: one or more recessive genes which are not linked to PMR genes, besides a closely linked or pleiotropic effect of one or more of the PMR genes in 'Profito'. Still, five lines with a significantly higher level of PMR than 'Corona' showed a significantly lower level of LCS than 'Profito'. Three of these lines, no's 7, 28 and 41 could hardly be distinguished from 'Corona' for the level of LCS. In experiment 2 this was confirmed,

where line 41 was even not different from 'Corona' for LCS. Although the PMR of these lines was only partial and lower than the PMR of 'Profito', it shows that lines with intermediate levels of PMR can have a very acceptable low level of LCS.

In a subsequent experiment, including the three F_1's that were obtained by intercrossing the three F_6 lines 7, 28 and 41, the commercially grown variety 'Flamingo', with an intermediate PMR, and the F_1's of 'Flamingo' × lines 7, 28 and 41, the results showed a similar genetic PMR for the lines 7, 28, and 41 and the variety Flamingo. However the LCS for the F_1's, obtained by intercrossing the F_6 lines 7, 28 and 41, was significantly lower than for Flamingo and the individual F_6 lines 7, 28 and 41 (results not presented). Possibly the lines 7, 28 and 41 are useful to breed chlorosis free varieties with intermediate PMR.

Although both 'Corona' and 'Profito' have been bred to minimize the expression of LCS, it is clear that a large variation in LCS occurs in their offspring. The PMR-LCS situation in cucumber resembles a similar case in barley, where the *ml-o* gene provides resistance to *Erysiphe graminis* DC. f.sp. *hordei* Em. Marchal and, as a pleiotropic effect, causes leaf chlorosis resulting in yield loss (Schwarzbach, 1976). By intensive recombination mildew resistant, high yielding and almost chlorosis free lines were obtained (Bjørnstad & Aastveit, 1990).

From the present study it is doubtful whether a high PMR can ever be combined with complete absence of LCS, when only PMR genes present in 'Profito' are used. If only a part of the resistance of 'Profito' is used, an intermediate level of PMR can be combined with a sufficiently low level of LCS to avoid yield loss. Use of the PMR gene(s) present in the three breeding lines 7, 28 and 41 will give an intermediate level of resistance with almost no LCS. Whether use of this level of partial PMR, possibly with help of biological pest control, will result in a sufficient reduction of the powdery mildew disease and fungicides is presently being tested at the Glasshouse Crops Research and Experimental Station (Naaldwijk, The Netherlands). Preliminary results of these tests are promising (Van Uffelen et al., 1992). If a higher level of PMR is required, this 'LCS free' PMR needs to be combined with newly found PMR genes (Zijlstra & Groot, 1992).

Acknowledgements

We thank Dr. K. Reining for critically reviewing the manuscript.

References

Bjørnstad, A. & K. Aastveit, 1990. Pleiotropic effects on the *ml-o* mildew resitance gene in barley in different genetical backgrounds. Euphytica 46: 217–226.

Brim, C.A., 1966. A modified pedegree method of selection in soybeans. Crop Sci. 6: 220.

Den Nijs, A.P.M. & D.L. Visser, 1980. Induction of male flowering in gynoecious cucumbers (*Cucumis sativus* L.) by silver ions. Euphytica 29: 273–280.

Groot, S.P.C., S. Zijlstra & J. Jansen, 1992. Phosphorus nutrition and selection against leaf chlorosis related to powdery mildew resistance in cucumber. J. Amer. Soc. Hort. Sci. 117: 500–503.

Jansen, R.C. & J. Jansen, 1990. On the selection for specific genes by single seed. Euphytica 51: 131–140.

Kooistra, E., 1968. Powdery mildew resistance in cucumber. Euphytica 17: 236–244.

Leppik, E.E., G. Sopwell, S.W. Braverman, S.M. Dietz & H.F. Winters, 1964. A summary of reports on the resistance of plant introductions to disease, insects and nematodes, *Cucumis sativus* and *Cucumis* spp.: 12–14.

Murashige & Skoog, 1962. A revised medium for rapid growth and bio assyays with tobacco tissue culture. Physiologia Plantarum 15: 473–497.

Schwarzbach, E., 1976. The pleiotropic effects of the *ml-o* gene and their implications in breeding. Barley Genetics III: 440–445.

Shanmugasundaram, S., P.H. Williams & C.E. Peterson, 1971. Inheritance of resistance to powdery mildew in Cucumber. Phytopathology 61: 1218–1221.

Straathof, Th.P., J.C. Jansen & H.J.N. Löffler, 1993. Determination of resistance to *Fusarium oxysporum* in *Lilium*. Phytopathology 83: 568–572.

Van Uffelen, J., L. Hogendonk & P. Steenbergen, 1992. Nieuwe methode onthult partiele resistentie. Prophyta 4: 26–29.

Zijlstra, S. & S.C.P. Groot, 1992. Search for novel genes for resistance to powdery mildew (*Sphaerotheca fuliginea*) in cucumber (*Cucumis sativus*). Euphytica 64: 31–37.